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Minireview

Stress molecules in sepsis and systemic inflammatory response syndrome

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Abstract During sepsis, microbial derived products ("pathogen-associated molecular patterns", PAMPs) are recognized as exogenous danger signals by specific sensors of the host ("pattern recognitions receptors", PRRs). This interaction leads to the release of numerous stress proteins that are a prerequisite to fight infection, though their overzealous production can contribute to tissue damage, organ dysfunction and eventually death. In critically ill patients, translocation of PAMPs can occur from the gut, and injured tissues and cells release endogenous danger signals called "alarmins" (e.g. High mobility group box-1); that share some properties with PAMPs. Thus, numerous similarities occur during infectious and non-infectious systemic inflammation.

Keywords: Cytokines; Endotoxin; Heat shock proteins; Interleukin-15; Synergy; TREM-1

1. Introduction: sepsis versus non-infectious systemic inflammatory response syndrome (SIRS)

During sepsis, a cascade of events is initiated by microorganisms and their derived products called "pathogen-associated molecular patterns" (PAMPs). These molecules are common to pathogenic, non-pathogenic, and commensal bacteria, making "microbial-associated molecular patterns" (MAMPs) a more appropriate term than PAMPs. They include surface molecules such as endotoxin (e.g. lipopolysaccharide, LPS; lipoproteins; outer-membrane proteins; flagellin; fimbriae; peptidoglycan, PGN; peptidoglycan-associated lipoprotein; and lipoteichoic acid, LTA), and internal motifs released following bacterial lysis (e.g. heat-shock proteins, HSP; DNA fragments). MAMPs are recognized by specific "pattern recognition receptors" (PRRs). In the host, Toll-like receptors (TLR) and cytoplasmic PRRs (e.g. NOD1 and NOD2) act as sensors of the MAMPs, recognized as exogenous danger signals. Within tissues, cells are exposed to more than one single signal, and multiple stimuli act in synergy leading to an enhanced production of inflammatory cytokines (Fig. 1). For example, a synergy has been reported between endotoxin and other microbial TLR agonists or Nod ligands, Gram-positive-derived exotoxins, viral infection, hypoxia, or simply glucose. Similarly, inflammatory cytokines (e.g. gamma-interferon, IFNγ; granulocyte–macrophage colony stimulating factor, GM-CSF; and tumor necrosis factor, TNF), inflammatory mediators (e.g. platelet-activating factor, PAF; substance P) further increase the LPS-induced macrophage activity and the LPS-induced lethality. Complement activation leading to anaphylatoxin C5a, or coagulation activation leading to thrombin further amplify cytokine expression. In most cases, these synergies lead to more severe organ failure.

Cytokines play a major role in orchestrating the anti-infectious process. Cytokines further enhance the microbicidal activities of phagocyting cells, contribute to the recruitment of leukocytes towards the site of infection, enhance hematopoiesis, and induce fever. Their production is very fast after the insult as illustrated by the TNF peak, only 1:30 h after injection of endotoxin. Many cells, including leukocytes, epithelial cells, endothelial cells contribute to these productions. Mast cells contain pre-formed cytokines, favoring a very rapid release of active molecules. While inflammatory cytokines contribute to the anti-infectious process, their excessive production has severe side effects. Anti-inflammatory cytokines are also produced in large amounts during sepsis. While their role is to dampen the inflammatory events, their excessive production may favor the immunodepression observed in sepsis. All these cytokines are part of a complex network of interactions and are integrated elements of the sepsis puzzle.

Non-infectious systemic inflammatory response syndrome (SIRS) shares many common parameters with sepsis. Critically ill patients in intensive care units (ICU) (e.g. resuscitated patients after cardiac arrest, vascular and abdominal surgery, trauma, burns...) undergo an inflammatory process that can lead to organ dysfunction and eventually death with pathophysiological events similar to those seen in sepsis. Matzinger revolutionized the definition of immunity by hypothesizing that immune system activity stemmed from recognition of and reaction to internal danger signals, rather than from discrimination between self and non-self molecules [1]. Most

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Abbreviations: ARDS, acute respiratory distress syndrome; GM-CSF, granulocyte–macrophage colony stimulating factor; HMGB-1, High mobility group box-1; HSP, heat shock protein; HDL and LDL, High and low density lipoproteins; ICAM-1, intercellular adhesion molecule-1; ICU, intensive care unit; IFNγ, gamma-interferon; IL, interleukin; LBP, LPS binding protein; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MOF, multiple organ failure; PAF, platelet-activating factor; PAMPs, pathogen-associated molecular patterns; PRRs, pattern recognitions receptors; SIRS, systemic inflammatory response syndrome; TLR, toll-like receptors; TNF, tumor necrosis factor; TREM-1, triggering receptor expressed on myeloid cells-1; VCAM-1, vascular cell adhesion molecule-1

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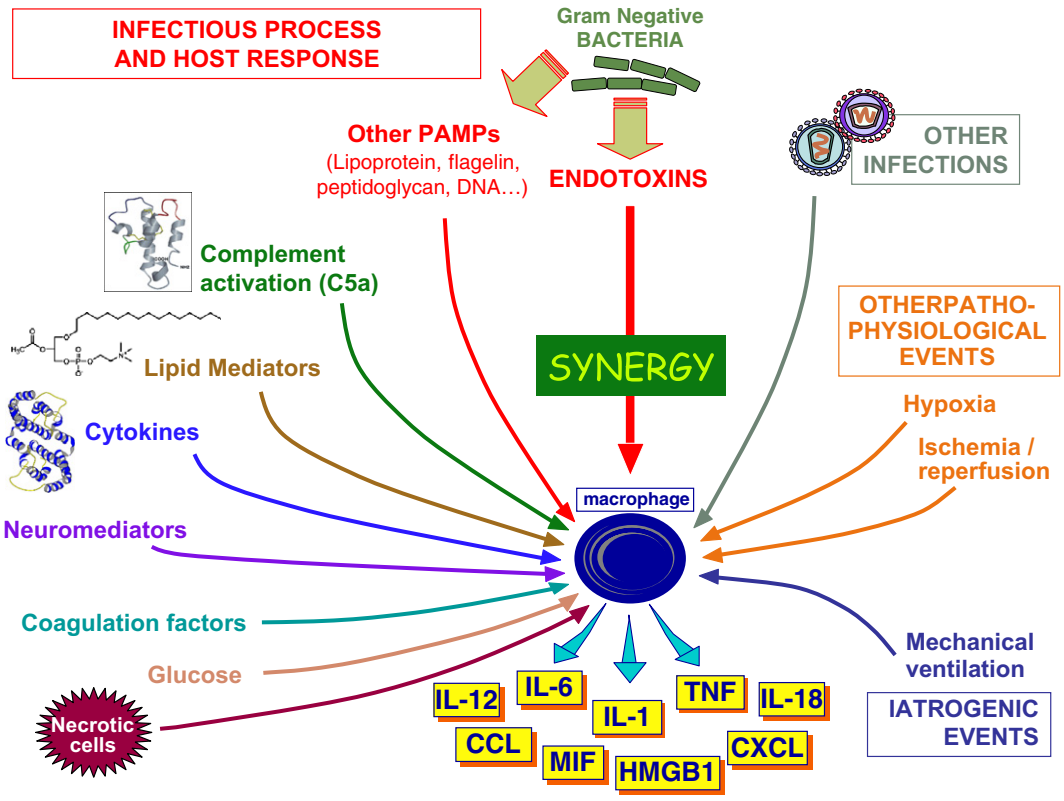

Fig. 1. Concomitant signals act synergistically. During an infectious process host's leukocytes (e.g. macrophages) are responding to exogenous danger signals that are the pathogens-associated molecular patterns (PAMPs). The response is amplified by endogenous mediators released during the anti-infectious response and by co-factors or concomitant stressful events, including endogenous danger signals (alarmins) or iatrogenic events.

fascinating is that similar PRRs can serve as receptors for internal danger signals generated by tissue injury (e.g. necrotic cells; released RNA; crystals of uric acid; high mobility group box-1, HMGB-1). The term "alarmin" has recently been proposed by Oppenheim to characterize such endogenous stress molecules that signal tissue and cell damage [2]. The possible translocation of endoxin from hypoxic gut can also contribute to the similar features that exist between sepsis and various SIRS.

2. Bio-markers of stress in plasma

2.1. Half-angel – half devil cytokines

During these last 20 years, the role of inflammatory cytokines to protect against infection has been well established in animal models. The different approaches included injection of recombinant pro-inflammatory cytokines, particularly TNF, interleukin-1 (IL-1), and IFNγ, injection of neutralizing antibodies directed against these cytokines, and the use of cytokine- or cytokine receptor-deficient mice. In humans, the altered capacity to produce IL-1 was associated with recurrent infections [3]. In the mean time, other models definitively demonstrated that lethal toxic shocks or lethal infections were due to the same cytokines, particularly TNF, IL-1β, IL-12, IL-18, IFNγ, GM-CSF, macrophage migration inhibitory factor (MIF), and IFNβ (Table 1). The lethality consecutive to the injection of endotoxin or superantigen, together with galactosamine, a hepatotoxic agent, was prevented in TNF receptor deficient mice [4]. However, it is worth noting that in the absence of galactosamine, similar amounts of LPS are required to kill TNF knock-out mice and normal mice [5]. On the other hand, anti-TNF treatments have been shown to be highly efficient in protecting animals against endotoxic shock and lethal bacteraemia. Such treatments also protected against pulmonary microvascular injury after intestinal ischemia injury, which is associated with endotoxin translocation [6].

IL-1β converting enzyme (ICE) or caspase-1 is the enzyme required for the maturation of the 30 kDa biologically-inactive IL-1β precursor to the mature 17 kDa active form of IL-1β. Interleukin-1β deficient mice are normally sensitive to the lethal effect of LPS, but the survival to a lethal dose of endotoxin reaches 70% among caspase-1-deficient animals [7]. These results most probably reflect the involvement of caspase-1 in the maturation of IL-18, another member of the IL-1 family. While IL-18 promotes healing from bacterial infection in mice, it accounts for both TNF and Fas Ligand-mediated hepatotoxicity in endotoxin-induced liver injury [8]. Neutralization of IL-18 protected mice against lethal *Escherichia coli* or *Salmonella typhimurium* endotoxemia [9] and IL-18 deficient mice showed decreased sensitivity towards LPS-induced shock [10]. IL-18 favors IFNγ production. In a cecal ligation and puncture (CLP) model of peritonitis, Echtenacher et al. [11] showed that concomitant injection of IFNγ further increased lethality. Similarly, a deleterious effect of IFNγ was demonstrated as anti-IFNγ treatment increased survival in a lethal infection with *Staphylococcus aureus* [12].

Table 1
Immune dysregulation during sepsis and SIRS is characterized by an exacerbated production of pro-inflammatory mediators that can lead to tissue injury and eventual lethality

Mediators that can contribute to tissue injury, organ or system dysfunction, and eventual lethality[a]	
Cytokines	Tumor necrosis factor (TNF) Interleukin-1 (IL-1) IL-2 IL-12 IL-15 IL-18 IL-27 Gamma interferon (IFNγ) IFNβ Granulocyte–macrophage colony-stimulating factor (GM-CSF) Leukemia Inhibitory factor (LIF) Macrophage migration inhibitory factor (MIF) *Some chemokines* CXCL8 (IL-8) CCL5 CXCR1 and 2 ligands CCR1 ligands CCR4 ligands[b]
Growth factors	Vascular endothelial growth factor (VEGF)
Cell markers of stress	High Mobility Group Box-1 protein (HMGB-1) Crystal of uric acid S100
Plasma factors	Ligand of Triggering receptor expressed on myeloid cells-1 (TREM-1) Anaphylatoxin C5a Mannose-binding lectin (MBL)
Lipid Mediators	Prostaglandins Leukotrienes Platelet activating factor (PAF) Oxidized phospholipids
Purine nucleoside	adenosine (via A_{2A} receptor)
Neuromediators	Substance P Noradrenalin
Enzymes	Cyclo-oxygenase-2 (COX2) 5-Lipoxygenase Phospholipase A2 Mast cell dipeptidyl peptidase I Elastase Glycogen synthase kinase-3 (GSK-3) Inducible nitrite oxide synthase (iNOS) Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase
Coagulation factor	Tissue factor Thrombin
Free radicals	NO O_2^-

[a]As demonstrated in animal models with the help of specific antibodies, inhibitors or antagonists, or with knockout mice.
[b]Either CCL17 or CCL22.

In patients, cytokines are produced in excess and are therefore detectable in blood, where they are normally absent. However, the circulating cytokines are merely the tip of the iceberg [13], and cell-associated cytokines can be shown even when plasma levels are undetectable. Among cytokines, IL-6 is probably the best marker of the severity of infectious or non-infectious stress. IL-6 induces a broad array of acute-phase proteins that limit inflammation, such as α-1-acid-glycoprotein or C-reactive protein. More recently, IL-1 receptor antagonist (IL-1Ra), LPS binding protein (LBP), and soluble CD14 were identified as acute-phase proteins. However, IL-6 may induce myocardial depression during septic shock, as shown during meningococcemia [14].

2.2. Inflammatory mediators

Sepsis is associated with increased plasma levels of histamine from mast cells and basophils following activation of complement pathways with up-regulation of anaphylatoxins C3a and C5a. Exogenous histamine or selective histamine H2 receptor agonist protect against endotoxin shock. Anaphylatoxins

enhance vascular permeability and smooth muscle contraction and are chemo-attractants for leukocytes. When compared to wild-type mice, C5-deficient mice respond to LPS by lower TNF levels and a lower severity index, and anti-C5a or anti-C5a receptor antibodies prevent death from sepsis. In contrast, knock-out mice for C4, C3, and C3 receptor are more susceptible to endotoxin, and C1 inhibitor confers protection against death from sepsis.

Pro-inflammatory cytokines induce synthesis of phospholipase A2, inducible cyclo-oxygenase (COX-2), 5-lipoxygenase, and acetyltransferase, which contribute to the synthesis of eicosanoids (prostaglandins and leukotrienes) and platelet-activating factor (PAF). These lipid mediators, acting through specific G-protein-coupled receptors, promote inflammation, altering vasomotor tone and increasing blood flow and vascular permeability. Mice deficient in phospholipase A2 receptor, or COX-2, or 5-lipoxygenase, are more resistant to endotoxin. However, prostaglandins E2 have also the property to reduce TNF production. A deleterious role has also been attributed to adenosine, an endogenous purine nucleoside that has been shown to contribute to the alteration of the immune status of septic patients, while the inactivation of its receptor (A_{2A}) increases survival in polymicrobial sepsis [15].

Produced by NADPH oxidase, superoxide anion oxidizes and alters proteins and unsaturated fatty acids of phospholipids. However, some oxidized phospholipids can prevent endotoxin-induced inflammation by blocking the interaction between LPS and LBP or CD14. Knock-out mice for NADPH oxidase compounds are more susceptible to severe infections, although their sensitivity to endotoxin remains unaltered. Mice deficient in inducible nitric oxide synthase (iNOS) merely exhibit less severe hypotension following lethal endotoxin challenge. In patients, NO is released in large amounts following endotoxin exposure and cytokine-related stimulation of iNOS activity within inflamed tissues and vessel walls [16]. This NO excess contributes to the development of microvessel damage, vascular hyporeactivity, and organ dysfunction, probably by inducing apoptosis.

Neuro-mediators play a major role in controlling inflammation. Substance P increases cytokine production, histamine release, leukocyte adhesion, chemotaxis, and vascular permeability. Catecholamines diversely interfere with cytokine production. Noradrenaline, via the α_2-adrenergic receptor, increases TNF production, whereas adrenaline interaction with the β_2-adrenergic receptor decreases TNF production in vitro and in vivo in LPS-challenged healthy volunteers, and concomitantly enhances IL-10 production. In addition, adrenaline increases IL-8 production and suppresses NO production. The anti-inflammatory effects of β-agonists are mediated through reduced IκBα degradation and through increased intracellular cAMP levels. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating peptide are two anti-inflammatory neuropeptides that inhibit cytokine production and protect mice from LPS lethality. In LPS-treated rats, vagal nerve stimulation attenuates hypotension and reduces plasma- and liver-TNF levels through an interaction between acetylcholine and the α7 subunit of the nicotinic receptor at the macrophage surface [17]. Finally, α-melanocyte stimulating hormone, is another neuro-mediator that dampens inflammation by inhibiting pro-inflammatory cytokine production.

Cross-talk between cytokines and neuro-hormones is the cornerstone of homeostasis restoration during stress. The production and release of vasopressin and corticotropin-releasing hormone are enhanced by circulating TNFα, IL-1, IL-6, and IL-2, by locally expressed IL-1β and NO, and by afferent vagal fibers. Moreover, cortisol synthesis is modulated by locally expressed IL-6 and TNFα. Up-regulated hormones help to maintain cardiovascular homeostasis and cellular metabolism and to wall-off foci of inflammation. Impaired endocrine responses to sepsis may result from cytokines, neuronal apoptosis, metabolic and ischemic derangements in the hypothalamic–pituitary and adrenal glands, and drug administration. Deficiencies in adrenal gland function and vasopressin production may occur in about one-half and one-third, respectively, of septic shock cases, contributing to hypotension and death.

2.3. Soluble membrane markers

Host serum contains several proteins that have been shown to interact with LPS. These proteins create a sensitive recognition system that allows the detection of trace amounts of this bacterial compound. Some of these proteins are present at homeostasis (such as sCD14), but their levels are considerably increased during sepsis [18]. Depending on their concentration, these LPS-binding molecules may facilitate LPS interaction with Toll-like receptor 4 (TLR4)-bearing cells, or on the contrary decrease cellular response. As an example, sCD14 has been shown to favor the response of cells devoid of membrane CD14, such as endothelial and some epithelial cells. In contrast, high levels of sCD14 compete with the membrane form present on monocytes, and inhibit monocyte response to LPS by transferring cell-bound LPS to plasma lipoproteins [18]. Thus, sCD14 reduces the ability of monocytes to produce cytokines in response to LPS, whereas, at the same time, it may enhance the response of endothelial or epithelial cells. The concentration of circulating sCD14 is modified not only during infection, but also after a major stress, such as trauma or surgery. Indeed, sCD14 concentration was found to increase postoperatively in patients undergoing surgery or after trauma. In addition to the blood compartment, an increase of sCD14 may be observed in the lungs of patients undergoing inflammatory or infectious processes. This is the case after acute respiratory distress syndrome (ARDS), sarcoidosis or tuberculosis.

The triggering receptor expressed on myeloid cells-1 (TREM-1) was originally described as a cell surface receptor selectively expressed on monocytes/macrophages and neutrophils (see below). Clinical studies reported that this molecule is present as a soluble form. Much interest was put on the latter form because it was found to be increased in the plasma of septic patients and in the bronchoalveolar lavage fluids of patients diagnosed with pneumonia [19]. sTREM-1 was thus considered to be a new and *specific* marker of infection. However, more recently, an increase of sTREM-1 was reported in the absence of infection, in the gastric juice of patients with peptic ulcer disease, and in pleural effusion in patients with neoplastic disorders [20,21]. Similarly, we were able to show high levels of sTREM-1 in the plasma of patients after surgical stress or ischemia-reperfusion [22]. Our study included two groups of patients without infection but with severe inflammation: elective heart surgery and patients resuscitated after cardiac arrest. In the cardiac arrest patients with refractory shock, sTREM-1

levels reached those found in the patients with severe sepsis. The increase of circulating sTREM-1 in non-infectious severe inflammation is probably due to bacterial translocation from the gut. It has been shown that the injection of LPS to healthy volunteers results in an increase of circulating sTREM-1 [23], and circulating endotoxin was detected in the plasma of many resuscitated cardiac arrest patients [24]. Thus, similarly to other molecules that were first thought to be specific to infection, e.g. procalcitonin or C-reactive protein, circulating sTREM-1 seems to be a marker of severe inflammation rather than infection.

Soluble forms of pattern recognition receptors or associated proteins may also be detected in human fluids. This is the case for soluble Toll-like receptor 2 (sTLR2). TLR2 belongs to a family of trans-membrane PPRs sensing bacterial products. TLR2 has been shown to sense bacterial lipoproteins, LTA [25], and eventually peptidoglycan although this latter property remains controversial. Soluble TLR2 was detected in human breast milk, saliva and serum. The biological role of this soluble receptor is not clear. It is thought that it may interfere and inhibit the binding of TLR2 ligands to the membrane form of this receptor and thus inhibit cell activation. The level of sTLR2 was found to be decreased in some pathologies such as tuberculosis and acute myocardial infarction, but there is no information about sTLR2 levels during sepsis. MD-2 is a soluble protein that is associated with TLR4 to form the receptor for LPS. MD-2 has been shown to be essential for TLR4 transfer from the Golgi apparatus to cell surface and a prerequisite for LPS sensing. Soluble MD-2 has been detected in the plasma of patients with severe sepsis or septic shock, and in lung edema fluids from patients with ARDS [26]. Similarly to sCD14, sMD-2 may enhance the reactivity of TLR4-positive epithelial cells towards LPS, whereas it would down-regulate the reactivity of cells positive for both TLR4 and MD-2, such as monocytes.

E-selectin, vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 are expressed on vascular endothelium. They are key molecules for leukocyte recruitment within the tissues. E-selectin is important for the rolling phase on vascular endothelium, and ICAM-1 and VCAM-1 are necessary for firm adhesion. The expression of E-selectin and ICAM-1 on endothelial cells is increased upon activation by cytokines or endotoxin. This increased expression leads to a massive recruitment of neutrophils and probably contributes to tissue damage and organ failure. In a mouse model of CLP an increase of ICAM-1 on lung endothelial cells has been shown [27]. More generally, infection and some inflammatory pathologies are associated with an increase of the soluble forms of these molecules. The mechanism of this induction is unclear, they are probably the result of membrane shedding after proteolytic cleavage. The precise role of these soluble molecules is also unknown. They may be induced to counteract the massive adhesion and recruitment of leukocytes on endothelial cells. However, the production of these soluble forms seems to be delayed as compared to their increased expression on endothelium. As an example, the maximum expression of ICAM-1 on lung endothelial cells is obtained 6 h post-CLP, whereas a significant increase of sICAM-1 in the plasma is only seen after 18 h [27]. Nevertheless, the levels of sE-selectin, sVCAM-1 and sICAM-1 are significantly increased during human sepsis and correlate with severity, multiple organ failure and poor outcome.

2.4. High and low density lipoproteins (HDL and LDL)

HDL and other plasma lipoproteins can bind and neutralize the bioactivity of Gram-negative bacterial LPS and Gram-positive bacterial LTA. During sepsis, circulating levels of HDL decline dramatically. Although it is unclear how the loss of HDL impacts inflammation and immune response, it is generally believed that the low levels of HDL impair the host's ability to neutralize LPS. However, when HDL levels decline in critically ill patients, LPS has been shown to bind preferentially to LDL and VLDL that maintain their ability to neutralize endotoxin [18]. In addition, the inhibitory effect of HDL is not always found, and this lipoprotein may also show pro-inflammatory properties. A recent study has shown that native HDL may in fact enhance monocyte response to LPS [28]. This enhancing effect was found in the presence of inhibitory concentrations of LPS-binding protein (LBP). This activity appeared to be due to the ability of native HDL to suppress the inhibitory activity of LBP. In contrast to native HDL, LDL and reconstituted HDL did not possess this activity.

2.5. Acute phase proteins

The circulating levels of many acute phase proteins (e.g. serum amyloid proteins, ferritin, mannose-binding protein, α2-macroglobulin), and the expression of coagulation factors (fibrinogen, plasminogen, thrombin, tissue factor) are enhanced during sepsis and SIRS. We will limit our discussion to the most "popular" acute phase protein, namely C-reactive protein and to LBP for its specific relevance to interact with endotoxin during sepsis.

C-reactive protein (CRP) is an acute phase protein, member of the pentraxin family, and produced by the liver. The pentraxins are found across invertebrate and vertebrate species and share a cyclic pentameric structure resistant to heat and proteases. CRP can bind to phosphocholine moieties and other ligands in a calcium-dependent manner. By interacting with polysaccharides and glycolipids on bacterial damaged membranes and exposed nuclear antigens, CRP induces the binding of C1q and the activation of the classical complement cascade. CRP has also been shown to bind to the Fc receptors and to enhance phagocytosis of microorganisms and cytokine production. Interestingly, CRP is not an acute phase protein in mice. However, CRP transgenic mice over-expressing rabbit CRP are protected against endotoxic shock [29]. CRP is mostly used to diagnose inflammation, as its levels rise dramatically during inflammatory processes, such as inflammatory bowel disease and some forms of arthritis, autoimmune diseases, myocardial infarction, atherosclerosis, and cardiovascular diseases. CRP levels were also found increased after major surgery and ischemia-reperfusion. The monitoring of CRP is often used in patients after surgery or other invasive procedures to detect the presence of an infection during the recovery period. Indeed, CRP concentration was found to be considerably increased during bacterial infection [30]. Thus, CRP is a multifunctional protein playing a role in inflammation and host defense.

LBP is present at homeostasis in the plasma, but its levels are considerably increased during sepsis [31]. This protein is mostly produced by the liver. In a concentration-dependent manner, LBP can potentiate cell response by favoring the interaction of LPS with the membrane form of CD14. Inversely, at high concentrations, it will inhibit cellular response,

by transferring LPS to HDL or LDL [32]. LBP plays also an important role in the lungs in response to LPS administration or bacterial infection. Lung epithelial cells have been reported to produce LBP in vitro, and pneumonia was associated with an increase of LBP concentrations in the bronchoalveolar lavage fluids of infected animals [33]. Furthermore, LBP knockout mice are more sensitive to the intranasal administration of high doses of LPS, and show an impaired defense against pneumonia, and intraperitoneal infections caused by Gram-negative bacteria [33]. This impaired host defense seems to be due to a delay in the inflammatory response and neutrophil recruitment on the site of infection. However, during i.v. injection of LPS, the absence of LBP did not have an impact on the in vivo inflammatory response of deficient mice [34]. This is probably due to the presence in the blood of other molecules (such as sCD14) having a role similar to that of LBP, and permitting an efficient cellular response to LPS.

2.6. Circulating endotoxin and markers of infection

It is worth mentioning that many of the severe insults that require admission of patients to ICU are associated with the presence of detectable amounts of endotoxin within the blood stream, independent of any infection. For example, plasma endotoxin has been found in 92% of the patients after cardiac surgery with cardio-pulmonary bypass, in 71% of the patients undergoing abdominal aortic surgery after clamp release, in 61% of the burn and trauma patients, in 57% of critically ill ICU patients, and in 46% of patients resuscitated after cardiac arrest [35]. The biological relevance of these levels of circulating endotoxin has been shown in different clinical settings. In resuscitated patients after cardiac arrest, the levels of circulating IL-6, IL-10 and IL-1 receptor antagonist (IL-1Ra) were higher among the patients with detectable circulating endotoxin [24]. In meningococcal disease, the plasma levels of LPS positively correlated with those of circulating chemokines [36].

In addition to translocated endotoxin, it is obvious, although poorly demonstrated, that other PAMPs can reach the blood stream. For example, Shaw Warren's group nicely demonstrated that bacterial peptidoglycan-associated lipoprotein could be detected in the blood of mice that underwent peritonitis [37]. Bacterial peptidoglycan has been detected in the blood of rats after hemorrhagic shock [38], and it is most probable that fragmented bacterial DNA could also be found in the blood compartment. This has recently been illustrated with the registration of the "Septifast" test that detects by polymerase chain reaction analysis the presence of bacterial or fungal DNA in blood: positive tests exceed positive blood cultures.

Failure of the gut barrier remains central to the hypothesis that endotoxin reaches systemic circulation via the portal route or via lymphatic vessels. Endotoxins escaping from the gut lumen contribute to activation of the host's inflammatory mechanisms, leading subsequently to tissue injury and multiple organ failure (MOF). In addition, local activation of the immune inflammatory system occurs, accompanied by a local production of cytokines and other immune inflammatory mediators [39]. These intestinal-derived mediators may result in a further exacerbation of the systemic inflammatory response. As stated by Swank and Deitch [40], "even if the immune inflammatory system, rather than the gut, is the "motor of" MOF, the gut remains one of the major pistons that turns the motor".

The plasma level of procalcitonin (PCT), the precursor of calcitonin, has been claimed to help to discriminate between infection and inflammation, to identify the occurrence of an infectious episode in critically ill patients, and to correlate with organ failure and mortality. However, other data have challenged the specificity of plasma PCT elevation as a marker for infection. In a recent review, it was suggested that the dynamics of PCT levels, rather than the absolute values, could be important in identifying patients with infectious complication after cardiac surgery [41]: PCT appears superior to C-reactive protein (CRP) in discriminating infections, and PCT levels increase markedly after bacterial and fungal infections, correlating with the severity of sepsis. As mentioned earlier sTREM-1 was also believed to be a good marker for bacterial infection, before high plasma levels were reported in non-infectious SIRS patients.

3. Cellular markers of stress

3.1. Nuclear and cytoplasmic markers

High-mobility group box-1 protein (HMGB-1) is a highly conserved (>95% identity between rodent and humans) nuclear protein that binds to cruciform DNA. It exists in a membrane ("amphoterin") and an extracellular form which interacts with plasminogen and tissue type plasminogen activator. HMGB-1 is released during sepsis and found in plasma. Higher levels are found among non-surviving patients [42]. It acts as an internal danger signal molecule or "alarmin". HMGB-1 is a late mediator of sepsis, it behaves like a cytokine, and stimulates the release of numerous pro-inflammatory cytokines by human monocytes, although this property has been recently questioned. Anti-HMGB-1 antibodies protect mice against sepsis even when delivered 24 h after the induction of sepsis (CLP model) [43]. Like endotoxin, it activates leukocytes through TLR4 as elegantly demonstrated in a model of liver ischemia reperfusion performed in wild-type and TLR4 knockout mice [44]. HMGB-1 also contributes to the ileal mucosal hyperpermeability in a model of hemorrhagic shock as demonstrated by the protective effect of anti-HMGB-1 antibodies [45].

Most interestingly, HMGB-1 seems to be the mediator that links during sepsis the occurrence of apoptosis and lethality [46]. Beneficial effects obtained in sepsis with drugs such as ethyl pyruvate are associated with a decreased expression of HMGB-1 [47].

Heat shock proteins (HSPs) are other "alarmins" released in different stressful situations (sepsis, trauma, major surgery, burns). As illustrated in Fig. 2, and in agreement with the literature, high levels of HSP70 were found in sepsis patients but significantly elevated levels were also found in brain death patients and in resuscitated cardiac arrest patients. In the latter group, levels were similar among survivors and patients who died after neurologic disorders, whereas significantly higher levels were measured in patients who died of a refractory shock. In these patients a correlation between HSP70 and endotoxin, IL-6, IL-8, sTREM-1 or soluble TNF receptor were observed. There was no correlation either with IL-10, lactates, clinical score (SAPS II), or the occurrence of infection. A lot of properties were attributed to HSP before it was realized that most were probably due to endotoxin contamination.

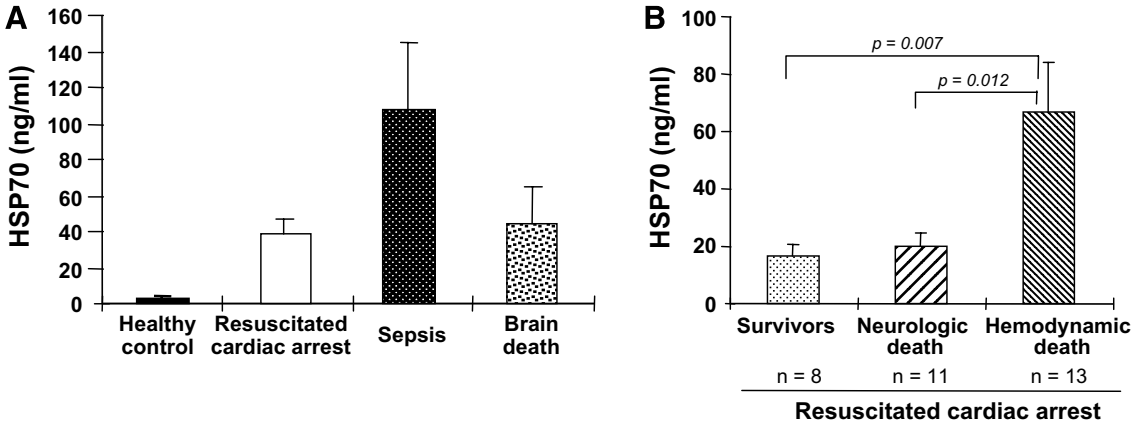

Fig. 2. Plasma levels of HSP70 in sepsis and SIRS patients. (A) Levels of heat shock protein 70 (HSP-70) measured by specific ELISA (Stressgen) in plasma of healthy controls ($n = 15$), resuscitated patients after cardiac arrest (RCA, $n = 32$), sepsis patients ($n = 4$) and brain death patients ($n = 11$). In RCA patients, HSP70 levels correlated with plasma levels of LPS ($r = 0.66$, $P = 0.01$), IL-6 ($r = 0.80$, $P < 0.0001$), IL-8 ($r = 0.9$, $P < 0.0001$), sTREM-1 ($r = 0.87$, $P < 0.0001$) and sTNFR ($r = 0.68$, $P < 0.0001$). (B) Levels of HSP70 in the plasma of RCA patients as a function of outcome.

However, there are convincing data that indicate that HSPs confer protection against ARDS, ischemia-reperfusion injury, and sepsis-induced lung injury. HSPs seem to dampen the inflammatory process as illustrated by the inhibition of LPS-induced cytokine production in human monocytes over-expressing HSP70 [48].

S100 proteins are small molecular size calcium-binding proteins. More than 20 members of this protein family have been described but three of them (S100A8, S100A9, and S100A12) are specifically linked to innate immune functions by their expression in the cytoplasm of phagocytes. They are released by phagocytes in response to cell stress. Some extracellular functions are related to anti-infectious host defense mechanisms, but the main characteristics of the phagocyte-specific S100 proteins are related to pro-inflammatory mechanisms. S100A8, and S100A9 induce a thrombogenic and inflammatory response in human endothelial cells. In addition, they induce a number of pro-inflammatory chemokines, as well as adhesion molecules like VCAM-1 and ICAM-1. They are significantly overexpressed at sites of inflammation, and there is a strong correlation between their serum concentrations and inflammation [49].

Monosodium urate crystals were recently identified as a "danger signal' released from dying cells. It was shown that the molecular mechanisms underlying monosodium urate crystals-induced inflammation engage the caspase-1-activating NALP3 inflammasome, resulting in the production of active IL-1β and IL-18 [50].

3.2. Cell surface markers on monocytes

In addition to the quantification of circulating cytokines or soluble molecules, immunodysregulation in sepsis or SIRS may be monitored by analyzing the expression of some cell surface molecules. Human leukocyte antigen-DR (HLA-DR) is one of these molecules. A profound decrease in its surface expression on monocytes has been regularly reported in sepsis. Low HLA-DR expression was associated with an increased risk of secondary bacterial infections [51], probably due to a less potent antigen presentation that would not allow an efficient adaptive immunity. The down-regulation of HLA-DR is at least partially mediated by the immunosuppressive cytokine IL-10 which was shown to favor the intracellular sequestration of this major histocompatibility complex type II molecule in human monocytes [52]. HLA-DR down-regulation was also observed in monocytes of patients with a non-infectious systemic inflammation, such as after pancreatitis, major surgery or trauma. We studied the expression of HLA-DR on monocytes from major trauma patients. Similarly to previous studies, we found a profound reduction of both the percent of HLA-DR positive monocytes and of the mean fluorescence intensity for trauma patients as compared to healthy volunteers (Fig. 3A and B) [53].

In addition to HLA-DR, we analyzed the expression of the membrane form of the pro-inflammatory cytokine IL-15. IL-15 shares with IL-2 several biological effects and IL-2 receptor β and γ chains. In contrast to IL-2 which is mainly produced by activated T cells, IL-15 is produced by a variety of tissues, including macrophages, dendritic cells, T-lymphocytes, or skeletal muscle. It is active on various cell types, including T and B lymphocytes, natural killer cells or neutrophils. IL-15 plays an important role in the development of natural killer cells and the production of IFNγ. The membrane form of IL-15 is found on normal monocytes and is able to stimulate T lymphocytes in vitro [54]. After ligation, membrane IL-15 may also induce a reverse signaling into the monocytes that results in the production of pro-inflammatory cytokines [55]. Similarly to HLA-DR, we found a decrease of the membrane form of IL-15 on monocytes of trauma patients (Fig. 3C and D). This reduced IL-15 expression may contribute to the lower monocyte and T cell pro-inflammatory response found in these patients. In contrast to the membrane form, circulating IL-15 was found to be increased in patients with severe melioidosis [56]. IL-15 levels were also increased in the serum of ARDS patients and correlated with poor outcome [57]. The latter observation contrasts with data obtained with mice transgenic for IL-15, that were found to be protected against a shock induced by *E. coli* infection [58]. In this animal model, the protective effect of IL-15 was due to an impairment of apoptosis. Indeed, multiple organ failure during sepsis is associated with an apoptotic state of monocytes/macrophages and lymphocytes [59]. IL-15 is a potent inhibitor of apoptosis and when increased it may protect against programmed cell death. However, at the same

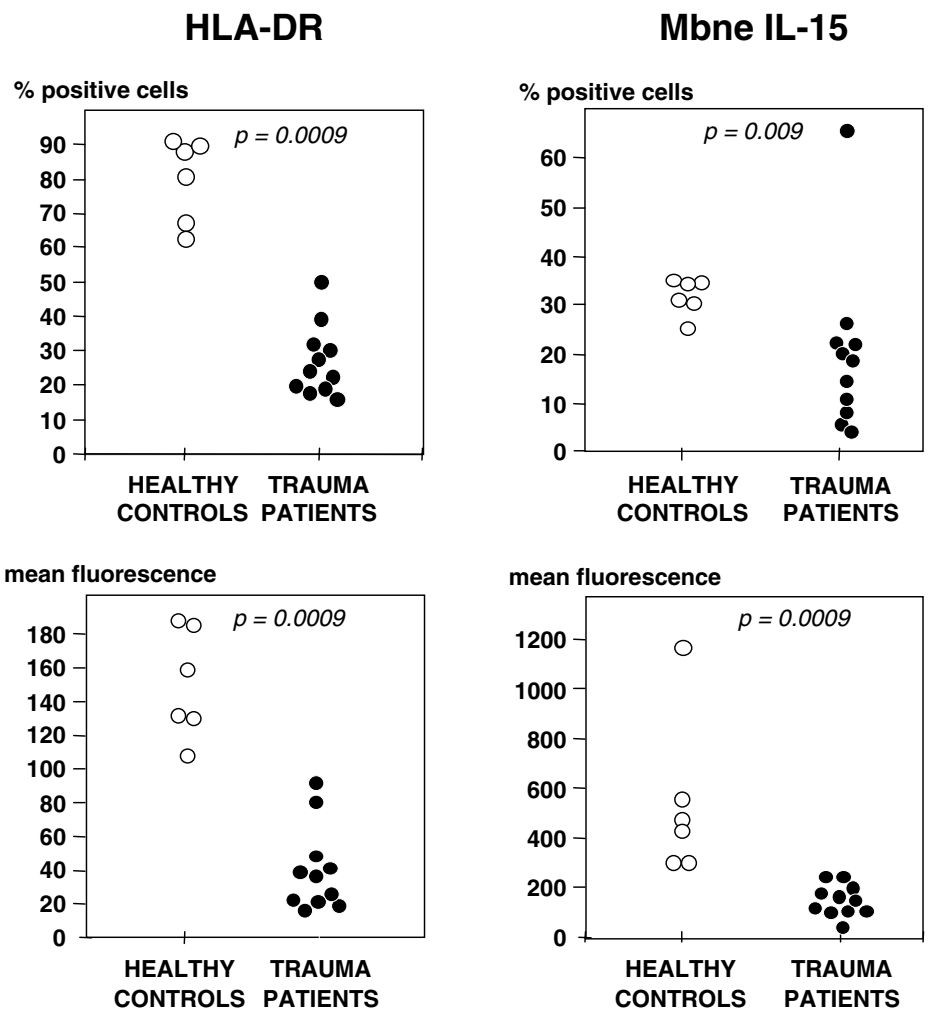

Fig. 3. HLA-DR and membrane IL-15 expression. HLA-DR membrane expression (left panels) and surface IL-15 expression (right panels) were assessed by flow cytometry onto CD14-positive circulating monocytes from healthy controls or trauma patients (anti-HLA-DR and anti-CD14 from Coulter; anti-human IL-15 from R&D systems). The results are expressed as percent of positive cells (upper panels) and mean fluorescence (lower panels).

time it is a pro-inflammatory cytokine that may synergize with IL-12 and amplify the inflammatory response leading then to organ failure and death [60]. It is not known whether there is a relationship between the increase in the soluble form of IL-15 and the decrease of its membrane form. Altogether, these results suggest that IL-15 may be an important cytokine to monitor during sepsis and severe inflammation. Although its precise role has not been clearly defined, IL-15 seems to be another marker of stress-induced immune dysregulation in critically ill patients.

TREM-1 is a receptor selectively expressed on monocytes/macrophages and neutrophils. TREM-1 belongs to the immunoglobulin superfamily, and consists of a single variable-type immunoglobulin domain ectodomain, a transmembrane domain and a short cytoplasmic tail. This cytoplasmic tail does not signal by itself, TREM-1 signaling needs the cooperation of the adapter molecule DAP12. The ligand of TREM-1 is still unknown but TREM-1 cross-linking with antibodies delivers a pro-inflammatory signal to the cell and synergizes with different bacterial products such as LPS or muramyldipeptide for pro-inflammatory cytokine production [61,62]. TREM-1 expression is increased on human monocytes upon LPS stimulation in vitro and blockade of TREM-1 protects mice against endotoxin shock [61]. As discussed above, this molecule also exists as a soluble form. The origin of this soluble form is not clearly defined. It may result from the proteolytic cleavage of the membrane form or be generated from an alternative splicing of the TREM-1 mRNA. Upon LPS injection in healthy volunteers, TREM-1 surface expression was found to be decreased on neutrophils, whereas it was increased on circulating monocytes [23]. In human sepsis, an increase of TREM-1 was found on patients circulating monocytes, but no change was noticed on neutrophils [63]. A slightly different result was found in a CLP mouse model of sepsis. In this model, TREM-1 surface expression was amplified for both circulating monocytes and neutrophils, as well as on macrophages and neutrophils present in the peritoneal cavity [63]. Thus, while it seems clear that LPS or bacterial infection result in an increased expression of TREM-1 on monocytes, the consequences for neutrophils seem less clear-cut. Very little is known about TREM-1 expression on monocytes during non-infectious severe inflammation. There is one study reporting an up-regulation of TREM-1 on patients' monocytes after major abdominal surgery [64].

In conclusion, markers of bacterial or non-infectious stress are found in large amounts in the plasma of sepsis and critically ill patients. These markers are danger signals delivered by the microorganisms and share many properties with internal danger signals (alarmins). For most of the stress proteins, their plasma levels correlate with severity and clinical scoring. In sepsis and SIRS, an exacerbated production of these mediators contributes to tissue damage, organ injury, alteration of the immune system, and eventually death. As recently demonstrated by Tang et al. about procalcitonin [65], a given marker can be appreciated for a while and dismissed later on. So far, IL-6 and surface HLA-DR expression are probably the best markers of the severity of the inflammatory process and of the altered immune status, respectively. However, they do not allow to discriminate between severe infection and systemic inflammation.

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